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Disruption of the interactions between the subunits of herpesvirus DNA polymerases as a novel antiviral strategy

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ABSTRACT

Most biological processes depend on the co-ordinated formation of protein–protein interactions. Besides their importance for virus replication, several interactions between virus proteins have been proposed as attractive targets for antiviral drug discovery, as the exquisite specificity of such cognate interactions affords the possibility of interfering with them in a highly specific and effective manner. There is a considerable need for new drugs active against herpesviruses, since available agents, most of which target the polymerisation activity of the virus DNA polymerase, are limited by pharmacokinetic issues, toxicity and antiviral resistance. A potential novel target for anti-herpesvirus drugs is the interaction between the two subunits of the virus DNA polymerase. This review focuses on recent developments using peptides and small molecules to inhibit protein–protein interactions between herpesvirus DNA polymerase subunits.

Keywords DNA polymerase, drug discovery, herpesviruses, polymerisation interactions, protein–protein interactions, review

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INTRODUCTION

Eight herpesviruses are known to infect humans, and several of these viruses are important human pathogens [1]. These viruses cause a wide variety of diseases. Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) cause herpes labialis and genitalis, keratoconjunctivitis and encephalitis; human cytomegalovirus (HCMV) is responsible for a variety of severe diseases in immunocompromised patients, including pneumonia, gastrointestinal disease and retinitis in transplant recipients and in AIDS patients, and is also a major cause of congenital defects in newborn children; varicella-zoster virus (VZV) is the causative agent of chicken pox following primary infection, but can reoccur in adults as herpes zoster (shingles); other members of the family include Epstein-Barr virus (EBV) and human herpesvirus 6, 7 and 8 (HHV-6, HHV-7 and HHV-8). Herpesvirus infections are increasing because of the growing number of immunocompromised individuals, i.e., transplant recipients and AIDS patients.

Antiviral agents licensed currently for the treatment of herpesvirus infections include acyclovir and derivatives, ganciclovir, foscarnet and cidofovir, all of which inhibit herpesvirus DNA polymerases [2]. Acyclovir, ganciclovir and cidofovir are nucleoside analogues which function as DNA chain terminators, whereas foscarnet inhibits virus DNA polymerase through binding to its pyrophosphate binding site. However, some of these antiviral agents, e.g., ganciclovir and foscarnet, can produce toxic side-effects. In addition, the emergence of virus strains resistant to commonly used anti-herpesvirus drugs is a growing problem, particularly in immunocompromised patients [3]. Therefore, there is still a great demand for the discovery of new, more effective and specific anti-herpesvirus agents.

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A novel strategy to inhibit virus replication is based on the disruption of virus protein–protein complexes by peptides or peptidomimetic compounds that mimic part of the interaction between subunits [4]. Many enzymes act as oligomer complexes, so inhibitors may act by preventing the formation of the active holoenzyme. Indeed, the first example in the literature of successful disruption of a protein–protein interaction involved an enzyme, the HSV-1 ribonucleotide reductase (RR). HSV-1 RR is a tetramer consisting of two large R1 subunits and two small R2 subunits [5]. In 1986, two independent groups of researchers reported that the synthetic nonapeptide YAGAVVNDL, corresponding to the C-terminus of the small subunit R2, could inhibit HSV-1 RR activity specifically by disrupting the interaction between the subunits [6,7]. Moreover, when the YAGAVVNDL peptide was linked to a protein carrier, the B subunit of *Escherichia coli* heat-labile enterotoxin, the resulting fusion protein inhibited virus replication and RR activity in HSV-1-infected cells specifically [8], thereby providing direct evidence of the antiviral efficacy of the YAGAVVNDL peptide in a cellular system. Thus, the YAGAVVNDL peptide became the first example of a new class of inhibitors that act by dissociating the subunits of multimeric enzymes.

Following these pioneering studies, a number of other peptides have been identified which disrupt protein–protein interactions between the subunits of other virus enzymes, e.g., the herpesvirus DNA polymerases. Replicative DNA polymerases generally function as multiprotein complexes, including a catalytic subunit and one or more accessory proteins that modify the properties of the core polypeptide. Analogously, it has been shown that a two-subunit DNA polymerase is a common theme among members of the Herpesviridae family, as the virus enzyme is composed of a catalytic subunit and an accessory protein, which is proposed to act as a processivity factor (Fig. 1). The best studied herpesvirus DNA polymerase is the HSV-1 polymerase, which is a heterodimer composed of two proteins, UL30 and UL42 [9]; in HSV-2, the infected cell-specific peptide (ICSP) 34,35 has been shown to be the counterpart of UL42 [10]. VZV polymerase interacts with a DNA-binding protein (encoded by gene 16) which also shows sequence similarity with UL42 [11]; in equine herpesvirus-1, proteins homologous to UL30 and

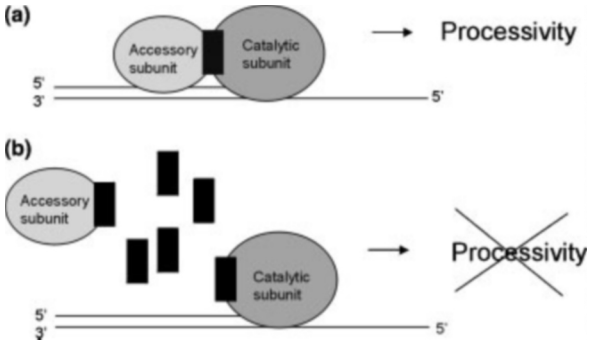


Fig. 1. Disruption of the herpesvirus DNA polymerase complex. (a) The DNA polymerase of herpesviruses is composed of a catalytic subunit, which possesses basal activity, and an accessory protein. By interacting with the catalytic subunit, the accessory protein stimulates the processivity of the enzyme. In both the HSV-1 and the HCMV DNA polymerase, the region of the catalytic subunit responsible for binding to the accessory protein has been localised to the C-terminal region (here represented as a rectangle). (b) Peptides corresponding to the C-terminal region of the catalytic subunit or small molecules mimicking the side-chain of residues crucial for subunit interaction are capable of disrupting the DNA polymerase complex, thus inhibiting the processivity of the virus enzyme.

UL42 are encoded by ORF30 and ORF18, respectively [12]. The HCMV DNA polymerase also consists of two proteins, UL54 and UL44 [13]. Other examples include the two subunits of pseudorabies virus DNA polymerase [14], the BALF5/BMRF1 complex of EBV [15], the catalytic subunit (Pol6) and the accessory protein (p41) of HHV-6 DNA polymerase [16,17], and the Pol8 and PF-8 subunits of HHV-8 DNA polymerase [18].

During the past few years, research interest has focused on the development of new anti-herpesvirus inhibitors which act by disrupting the interaction between the subunits of herpesvirus DNA polymerases, i.e., HSV-1 and HCMV DNA polymerase. Efforts have been aimed at characterising protein–protein interactions between the enzyme subunits, and at identifying peptides and small molecules that mimic either face of the subunit interaction, and which are therefore able to disrupt the virus protein complexes.

Herpes simplex virus DNA polymerase

The HSV-1 DNA polymerase is a heterodimer composed of a catalytic subunit, Pol or UL30, and an accessory protein, UL42, which stimulates the

processivity of the enzyme [19,20] without increasing the rate of catalysis per primer-binding event [21]. Mutations of UL30 and UL42 which disrupt the interaction between the two enzyme subunits specifically were shown to inhibit virus replication, thus supporting the essential role of the UL30/UL42 association [22–26]. This has led to growing interest in the potential of this association as a novel antiviral drug target for rationally designed compounds that, by mimicking the protein interface, would prevent heterodimerisation.

For this reason, the identification of the UL30 and UL42 regions responsible for the physical and functional interaction of these two proteins has been the subject of detailed investigation. For UL42, initial attempts were unsuccessful [27]. It was then shown that the N-terminal two-thirds of the 488-residue UL42 protein are sufficient for both binding to, and stimulation of, UL30 [23,28–30]; indeed, the first 338 residues are sufficient for HSV-1 replication [23,28]. On the other hand, the amino-terminal 20 amino-acids were shown not to be required for either binding or stimulation of UL30 [23]. Finally, random peptide display studies, coupled with mutational and calorimetric analyses, identified residue Q171 as important for binding to UL30 [31]. This residue lies within the so-called 'connector loop', a long loop which connects the two topologically similar domains of UL42. Indeed, the Q171A substitution in UL42 drastically reduced both binding to, and stimulation of, long-chain DNA synthesis by UL30 [31]. A co-crystal structure of UL42 bound to a UL30 C-terminal peptide then showed the presence of a hydrogen-bonding network which connects Q171 to the side-chain of UL30 residue R1229 [32].

The region of UL30 responsible for the interaction with UL42 was localised initially to the C-terminal 227 amino-acids [33]. Subsequent investigations to identify the essential regions demonstrated that the extreme C-terminus of UL30 is necessary and sufficient for specific interaction with UL42 [22,25,26]. It is noteworthy that this region of UL30 is not highly conserved among any other herpesvirus or cellular DNA polymerase, and that it is not necessary for catalytic activity, making it particularly attractive as a starting point for the de-novo design of new specific anti-HSV drugs. Attempts to disrupt the interaction of UL30 with UL42 resulted in the identification of peptides corresponding to the last 36, 27 and 18 residues of UL30 (Fig. 2), which inhibit the ability *in vitro* of UL42 to stimulate both the rate of DNA synthesis and the synthesis of long DNA chains by UL30, with IC_{50} values of 2–30 μM [34,35]. Moreover, an oligopeptide corresponding to the 27 C-terminal amino-acids of UL30 inhibits virus replication with an EC_{50} of 11 μM when delivered into HSV-1-infected cells via a protein carrier (the B subunit of *E. coli* heat-labile enterotoxin) [36]. It was proposed that the antiviral activity of this 27mer, designated as 'Pol peptide', occurred through the specific intracellular dissociation of

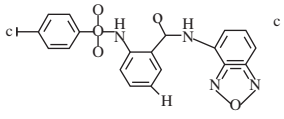
Peptide/ Small molecule	Target	*In vitro* activity IC_{50} (μM)	Activity in cell culture EC_{50} (μM)	Cytotoxicity CC_{50} (μM)
DDVAAPLPAAOFOAVOAOATAEETRRNLHRAFDTLA[a]	HSV-1 DNA polymerase	2	n.d.	n.d.
ADFGAVGAGATAEETRRNLHRAFDILA[b]	HSV-1 DNA polymerase	25	11	>200
ATAEETRRLHRAFDTLA[a]	HSV-1 DNA polymerase	30	n.d.	n.d.
 [c]	HSV-1 DNA polymerase	15	0.3-2	20
LDPRMHLHLEPALPYVEAHECC[d]	HCMV DNA polymerase	20	n.d.	n.d.

Fig. 2. Inhibitory peptides or small molecules directed against subunit interactions of herpesvirus DNA polymerases. n.d., not determined. References: [a][35]; [b][34,36]; [c][40]; [d][52].

the complex between UL30 and UL42 [36]. In addition, the structure of Pol peptide, both alone [37] and bound to the protein carrier [38], was elucidated and a nuclear localisation signal was identified in the inhibitory peptide [39].

Taken together, these studies established proof-of-principle for blocking the UL30/UL42 interaction as an antiviral strategy. The results also suggested that Pol peptide, or peptidomimetic derivatives, merit consideration as lead compounds for the development of novel anti-HSV therapeutic agents. In addition, biophysical studies of the UL30/UL42 binding interface demonstrated that, although numerous hydrophobic interactions are observed in the co-crystal structure of UL42 bound to a UL30 C-terminal peptide [32], only a few specific hydrogen bonds are crucial determinants of binding energy. Indeed, single amino-acid changes, i.e., substitutions at positions corresponding to UL30 residues 1228 and 1229, or at UL42 residue 171, can disrupt the UL30/UL42 interaction [31]. These residues are involved in hydrogen bonds crucial for the UL30/UL42 interaction [32]. Combined, these findings suggested that disruption of a very few bonds would be sufficient to disrupt the interaction between the two proteins, and that small molecules targeting the relevant side-chains could interfere with UL30/UL42 binding. This suggestion was supported by the identification, from a library of *c*.16 000 small molecules, of a compound, termed BP5 (Fig. 2), which inhibits the physical interaction between UL30 and UL42, and thus virus replication, specifically at concentrations (0.3–2 μM) below those that cause cytotoxic effects (20 μM) [40].

Human cytomegalovirus DNA polymerase

HCMV DNA replication has not been as well-characterised as that of HSV; however, HCMV homologues of six of the seven proteins essential for HSV-1 replication [41] have been identified [42]. These homologues include the two subunits of the DNA polymerase, which is composed of a 1242-residue catalytic subunit, Pol or UL54 [43,44], and a 433-residue accessory protein, UL44 or infected-cell protein 36 (ICP36) [13]. The HCMV DNA polymerase has a number of characteristics in common with its HSV-1 counterpart. The UL54 protein, which is the homologue of HSV-1 UL30, possesses DNA-dependent DNA polymerase activity [45] as well as 3′-5′ exonuclease activity [46]. Both these activities are dependent on the salt concentration [47], in a manner very similar to that observed for the HSV-1 DNA polymerase [48]. The HCMV UL44 accessory subunit is analogous to the HSV-1 UL42 protein. Like UL42, UL44 has been shown to bind double-stranded DNA with high affinity, to specifically interact with UL54, and to stimulate long-chain DNA synthesis, possibly by increasing the processivity of the polymerase along the DNA template [13,49]. Although the HCMV accessory protein has little sequence homology to UL42 and to the accessory proteins of other herpesviruses, it has been predicted that it possesses a similar 'processivity fold' structure [32].

The observations that both UL54 and UL44 are essential for HCMV DNA replication [42,50], that inhibition of UL44 synthesis in HCMV-infected cells inhibits virus DNA replication strongly [51], and that the UL54/UL44 interaction is specific [52], suggested that such an interaction might be an attractive target for antiviral drugs. However, in contrast to the relatively detailed understanding of the HSV-1 UL30/UL42 interaction, the interaction between the two subunits of HCMV DNA polymerase, UL54 and UL44, has taken longer to elucidate.

To characterise the HCMV DNA polymerase, the two subunits, UL54 and UL44, have been expressed and purified from insect cells infected with recombinant baculoviruses [52]. In order to localise the UL44-binding site on UL54, overlapping peptides spanning the C-terminal region of UL54 were synthesised and tested for inhibition of the UL54/UL44 interaction by two independent methods [52].

First, by means of an interaction ELISA, it was found that a peptide corresponding to the C-terminal 22 amino-acids of UL54 (residues 1221–1242) blocked the physical interaction between Ul54 and UL44. The concentration of peptide required to obtain 50% inhibition was *c*.11 μM (Fig. 2), a value comparable with that observed (2–30 μM) for inhibition of the HSV-1 UL30/UL42 interaction by UL30 C-terminal peptides [34,35]. Interestingly, the UL54 C-terminus presents two carboxy-terminal cysteines, which are uncommon among herpesvirus DNA polymerases and are important for the inhibitory activity of the peptide. Removal of one cysteine from the extreme C-terminus of the inhibitory peptide

diminished the capability of the peptide to interfere with the physical interaction between UL54 and UL44, while deletion of the two cysteines impaired the inhibitory activity of the peptide substantially [52]. These results suggested that these residues of UL54 might be involved in the interaction with UL44, either by binding UL44 directly, or by stabilising a structure within UL54 itself that can bind subsequently to UL44.

Second, the ability of the peptides spanning the UL54 C-terminal region to inhibit enzyme activity *in vitro* was analysed. The results demonstrated that the C-terminal 22mer inhibits the UL54/UL44 functional interaction efficiently. The concentration of C-terminal peptide required to inhibit UL54/UL44 activity by 50% was very similar to the IC_{50} for inhibition of the physical interaction (20 μM vs. 11 μM) (Fig. 2). Inhibition of the HCMV UL54/UL44 activity by the UL54 C-terminal peptide appeared to be specific, since the peptide did not inhibit the HSV-1 UL30/UL42 interaction at a concentration as high as 1 mM, and a peptide corresponding to the 27 C-terminal residues of HSV-1 UL30 (aa 1209–1235) did not inhibit the HCMV UL54/UL44 interaction [52]. Importantly, these findings demonstrated that successful, specific inhibition of HCMV DNA polymerase can indeed be obtained through disruption of the interaction between the two enzyme subunits.

In addition, these data suggested that the extreme C-terminus of UL54 might be involved in the interaction with UL44. To address this hypothesis, the binding to UL44 of peptides corresponding to the extreme C-terminus of UL54 was measured quantitatively using isothermal titration calorimetry (ITC). A peptide corresponding to the last 22 residues of UL54 was sufficient to bind specifically to UL44 in a 1 : 1 complex with a dissociation constant of *c*.0.7 μM [53]. Moreover, the deletion of this segment from UL54 prevented binding to UL44 [53]. These results demonstrated that this relatively small region of UL54 is both necessary and sufficient for UL44 binding. Conversely, a shorter peptide, corresponding to the last 10 residues, exhibited no detectable binding to UL44 in solution, and no detectable inhibitory activity against DNA synthesis by UL54 and UL44. Thus, this shorter region of UL54 is not sufficient for the interaction with UL44. However, although the C-terminal 10-residue segment is not sufficient for UL44 binding, the extreme C-terminus of UL54 is involved in the UL54/UL44 interaction, as deletion of the two C-terminal cysteines reduced binding of the UL54 C-terminal peptide and of full-length UL54 to UL44 [53].

These findings demonstrated clearly that the extreme C-terminus of HCMV UL54 is crucial for binding UL44, as is the C-terminus of HSV-1 UL30 for its interaction with UL42. Remarkably, despite this functional analogy of the C-terminus of UL54 to that of UL30, there is almost no sequence homology, and in fact, neither the HSV-1 nor the HCMV catalytic subunits are stimulated by the non-cognate accessory protein. In addition, other features of the interaction between UL54 and UL44 that are reminiscent of the interaction between UL30 and UL42 have been highlighted. First, the C-terminal region of UL54 can fold into a helical structure, as already reported for the C-terminus of HSV-1 UL30 [32,54]. In fact, circular dichroism (CD) spectroscopy of the UL54 C-terminal peptide in 2,2,2-trifluoroethanol (TFE)/water indicated that this peptide has some propensity to adopt a partially α-helical structure [52]. The helicity of the peptide was apparently independent of peptide concentration, thereby suggesting that it is monomeric, as was found for the C-terminal UL30 peptides [35]. Second, a nuclear localisation signal, RRLHL, was identified within the C-terminal region of HCMV UL54, and a functionally equivalent sequence, corresponding to residues RRMLHR, was found also in the C-terminus of HSV-1 UL30 [39]. Third, like HSV-1 UL42, HCMV UL44 is a DNA-binding protein [55] that is essential for virus replication [42,50], associates specifically with the catalytic subunit, and stimulates long-chain DNA synthesis [13,49]. The UL54- and DNA-binding activities of UL44 reside within the N-terminal two-thirds; as for UL42, the C-terminus is dispensable [49].

To further characterise the UL54/UL44 interaction, individual residues in UL54 that are crucial for binding to UL44 were defined. For this purpose, a series of mutations was engineered in the C-terminal region of UL54, and the effect of the mutations was tested on physical and functional interaction between UL54 and UL44 [53]. Initial studies focused on the last two residues of UL54, as previous studies had shown that a mutant UL54 C-terminal peptide lacking the two carboxy-terminal cysteines was >40-fold less potent than the wild-type peptide for disruption

of the physical interaction between UL54 and UL44, suggesting an important role for these residues in the UL54/UL44 interaction [52]. The results of mutational analysis demonstrated that the two C-terminal cysteines of UL54 probably play a role in the UL54/UL44 interaction, but not an essential one. In fact, a mutant UL54 peptide lacking the two C-terminal cysteines still bound UL44 in solution, although with an affinity lower than that of the wild-type peptide [53]. Consistent with these data, the deletion of these two residues in full-length UL54 caused only a partial impairment in the ability of UL54 to interact with UL44 in glutathione S-transferase (GST)-pulldown assays. Moreover, substitution of either or both the C-terminal cysteines with alanine did not cause any significant effect on binding of UL54 to UL44. These findings suggested that interactions between UL44 and the extreme C-terminus of UL54 probably involve the main chain of the two C-terminal cysteines rather than their side-chains. Similarly, the crystal structure of the HSV-1 UL42/UL30 peptide complex revealed that the last two residues of the UL30 peptide, Leu1234 and Ala1235, interact with UL42 via hydrogen bonds between the main chain carbonyl oxygens and the amino-group of the Lys289 side-chain of UL42 [32].

Next, 'alanine scan' mutants of UL54, in which each non-alanine residue in the region corresponding to the last 22 residues of UL54 was individually converted to an alanine, were created and tested for their ability to bind UL44 [53]. Substitution of Leu1227 or Phe1231 in UL54 impaired greatly both the UL54/UL44 interaction in pulldown assays and long-chain DNA synthesis, but without affecting basal polymerase activity, thereby identifying these residues as important for subunit interaction [53]. Thus, these observations highlight both similarities and differences between the UL54/UL44 and UL30/UL42 interactions. Both in HSV-1 and in HCMV DNA polymerase, a few residues are crucial for the binding of the catalytic subunit with the cognate accessory protein. However, the side-chains of the residues that have been identified as important for HSV-1 UL30/UL42 and for HCMV UL54/UL44 interaction are remarkably different, being basic in the first case and hydrophobic in the latter. In fact, although two basic residues of UL54, Arg1224 and His1226, also seem to participate in UL44 binding, they are not crucial [53]. As both the Leu1227-to-Ala and the Phe1231-to-Ala change substituted a larger side-chain for a smaller one, while maintaining the hydrophobic character of the residue, it can be speculated that large side-chains at these positions in UL54 are necessary to make intermolecular contacts with UL44. The importance of an aromatic hydrophobic side-chain at position 1231 of UL54 for UL44 binding is suggested by the observation that substitution of Phe1231 with a tyrosine restores both binding to UL44 in pulldown assays and long-chain DNA synthesis by UL54 in the presence of UL44 [53].

The crystal structure of residues 1–290 of UL44 has been elucidated to a resolution of 1.85 Å by multiwavelength anomalous dispersion [56]. The crystal structure of UL44 revealed an overall fold that was strikingly similar to that of UL42, despite the fact that the HCMV accessory protein has very little sequence homology to UL42. Moreover, as well as UL42, UL44 possesses a 'connector loop', a structural element that connects two topologically similar domains of the protein [32]. In UL42, the connector loop has been shown to be crucial for interaction with its cognate catalytic subunit, UL30 [31].

To investigate whether the UL44 connector loop might be involved in the interaction with UL54, each of its amino-acids (aa 129–140) was substituted with alanine [57]. The effect of each substitution on the UL44/UL54 interaction was then tested using GST-pulldown and ITC assays, as well as the effect on the stimulation of UL54-mediated long-chain DNA synthesis by UL44, and on the binding of UL44 to DNA-cellulose columns. Substitutions that affect residues 133–136 of the connector loop impaired the UL44/UL54 interaction measurably, but without altering the ability of UL44 to bind DNA. One substitution, I135A, disrupted the binding of UL44 to UL54 completely and also inhibited the ability of UL44 to stimulate long-chain DNA synthesis by UL54. Thus, similar to the UL30/UL42 interaction, a residue of the connector loop of the accessory subunit is crucial for UL54/UL44 interaction. However, although residue(s) of both UL44 and of UL42 that are important for binding to the cognate catalytic subunit reside in the connector loop, it remains to be determined whether the role played by these residues is similar or different in the two systems.

Two observations hint at differences. First, weak binding of UL30 could be detected with

the UL42 Q171A mutant in maltose binding protein (MBP)-pulldown assays, and a small release of heat was measured by ITC when a large excess of an UL30 C-terminal peptide was added to the Q171A mutant [31]. In contrast, the I135A substitution of UL44 impaired binding to UL54 in GST-pulldown assays completely and also reduced the affinity for UL54 C-terminal peptide to unquantifiable levels in ITC experiments. Second, Q171 is a polar residue while I135 is nonpolar.

Thus, although the residues most important for DNA polymerase subunit interactions lie in analogous regions, i.e., the C-terminus of the catalytic subunit and the connector loop of the accessory protein, the molecular details of the HCMV UL54/UL44 interaction are likely to be different from those of the interaction between its HSV counterparts. In particular, the UL44/UL54 interaction is likely to be more dependent upon hydrophobic interactions, since two of the residues of the UL44 connector loop that are most important for binding to UL54 (I135 and V136) are hydrophobic. Similarly, UL54 residues important for UL44 binding are hydrophobic [53], whereas those of UL30 which are important for interaction with UL42 are basic [31,32]. Consistent with this idea are the ΔH values, which are higher for the HCMV interaction than for the HSV-1 interaction [31,53], and may relate to the more crucial role of hydrophobic vs. hydrophilic residues in the two systems. This contrasts with the UL30/UL42 interaction, where a few specific hydrogen bonds between polar residues comprise the crucial sequence-specific determinants for binding [31,32].

The differences between the HCMV and HSV DNA polymerase subunit interactions probably account for the fact that these interactions are specific, since non-cognate partners do not bind [52]. As the residues most important for HSV-1 UL30/UL42 and for HCMV UL54/UL44 interaction are not conserved, small molecule inhibitors that target the residue side-chains could be significantly more virus-specific than most of the drugs currently licensed for antiherpesvirus chemotherapy. In support of this hypothesis, some small molecules able to block the UL54/UL44 interaction specifically *in vitro*, as well as HCMV replication, have been identified (A. Loregian and D. M. Coen, unpublished results).

Another potentially interesting antiviral target could be the UL44 protein itself. Indeed, the crystal structure revealed that, despite the fact that UL44 has an overall fold strikingly similar to that of HSV-1 UL42, UL44 can form a C-clamp-shaped dimer [56], whereas UL42 is a monomer both in solution and in the crystal structure [32,58]. These findings suggest that HSV and HCMV processivity factors adopt different oligomeric states during virus DNA replication. UL44 dimerises across one of the crystallographic two-fold axes, and the interaction between the two monomers entails an antiparallel β interaction, which involves six main-chain-to-main-chain hydrogen bonds and extensive packing of hydrophobic side-chains at the interface. F121 of each monomer is buried against a hydrophobic loop composed of P85, L86 and L87 of the other monomer. L86 and L87 also pack against M123 and L93 of the opposite monomer. These interactions entail a fairly large interface (*c*.1100 Å). Both the nature and the extent of the interactions suggest that the dimerisation is likely to be biologically relevant and not an artefact of crystallographic packing. Indeed, analytical ultracentrifugation and gel filtration measurements demonstrated that UL44 also forms a dimer in solution [56]. Sedimentation equilibrium analysis was also employed to determine a dissociation constant for UL44 dimerisation of 243 ± 63 nM [56].

To confirm that the residues identified in the dimer interface of the UL44 crystal structure are indeed crucial for UL44 homodimerisation, three mutant constructs, F121A, L86A/L87A and P85G, were created [56]. The F121A and L86A/L87A mutations were predicted to affect UL44 dimerisation, as these residues make extensive contacts with hydrophobic residues along the dimer interface. In contrast, the P85G substitution was expected to have a minor or no effect, as the P85 residue is not buried so significantly at the dimer interface. Indeed, gel filtration experiments demonstrated that the P85G mutant behaves as a dimer in a similar way to the wild-type protein, while the F121A and L86A/L87A mutants behave as monomers.

Finally, in order to determine whether dimerisation of UL44 affects DNA binding, the ability of the UL44 mutants that are altered at the dimer interface to bind DNA was investigated [56]. The mutant P85G, which could dimerise, displayed an affinity for DNA similar to that of the wild-type protein. In contrast, the F121A and L86A/L87A mutants, which behaved as monomers in gel

filtration experiments, had affinities for DNA that were 10- to 100-fold lower than that of the wild-type protein. These data suggest that the dimerisation of UL44 could play an important role in its activity as a DNA polymerase processivity factor, and therefore that the UL44 homodimeric interface may be exploited in the design of novel antiviral agents.

CONCLUSIONS

The importance and specificity of protein associations in virus replication and pathogenesis make protein–protein interactions attractive targets for therapeutic intervention. Compounds capable of interfering selectively with such interactions represent novel potential agents for antiviral therapy. However, although the disruption of specific protein–protein contacts is a promising strategy for drug development, the nature of these interactions can make this goal impractical. Many protein–protein interactions involve large surfaces or multiple contacts, making it unlikely that a single small molecule could interfere with them.

Recent studies on herpesvirus DNA polymerases, i.e., those of HSV-1 and HCMV, have identified important aspects of the subunit interface. In both the HSV-1 UL30/UL42 and HCMV UL54/UL44 interactions, only a relatively small surface appears to be necessary and sufficient for binding between the catalytic subunit and the cognate accessory protein; indeed, the substitution of single residue side-chains is sufficient to disrupt the subunit interaction and to inhibit the activity of the holoenzyme. These findings herald the prospect that inhibitors targeting the residue side-chains could interfere with the HSV-1-UL30/UL42 or HCMV UL54/UL44 interactions. Moreover, as the side-chains of the residues that are crucial for UL30/UL42 and UL54/UL44 interaction are remarkably different, such inhibitors could be significantly more virus-specific than most of the drugs currently licensed for antiherpesvirus chemotherapy, and would therefore represent novel, attractive antiviral compounds.

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